INFLUENCE OF LIPID CONTENT OF A LIPOSOME FORMULATION USED FOR SOLUBILIZATION OF A POORLY WATER-SOLUBLE DRUG ON ABSORPTION THROUGH CACO-2 MONOLAYERS

Schneider, M. and Imanidis G.

Institute of Pharmaceutical Technology, University of Basel, Klingelbergerstrasse 50, 4056 Basel, Switzerland

marcel.schneider@unibas.ch

Summary

• The purpose of this work was to determine the influence of liposomes used to solubilize poorly water soluble drugs on transport processes through Caco-2 cell monolayers.

•The influence of different lipid concentrations was investigated with progesterone as a passively absorbed model drug with a high permeability.

• Progesterone was incorporated in phosphatidylcholine liposomes produced by film method and extrusion through polycarbonate filters.

•The drug absorption was determined in a bi-directional Caco-2 assay, using purely aqueous drug solutions as reference. The cells (passage 60-65) were grown on Transwell inserts for 19-21 days. The drug quantification was performed by HPLC/MS.

•A mathematical model was developed that took into account transport between the apical, the cellular and the basal compartment and expressed passive cell membrane permeation by the apparent permeability coefficient.

•The apparent permeability coefficient, P_{app}, was calculated by numerical solution of the differential equations (see Equations 1-7) and simultaneous fitting to apical and basal concentrations of both transport directions with the software EasyFit®.

•P_{app} for a given lipid concentration was independent of drug concentration as also observed in the aqueous solution, but strongly dependent on the liposome-water distribution of Progesterone.

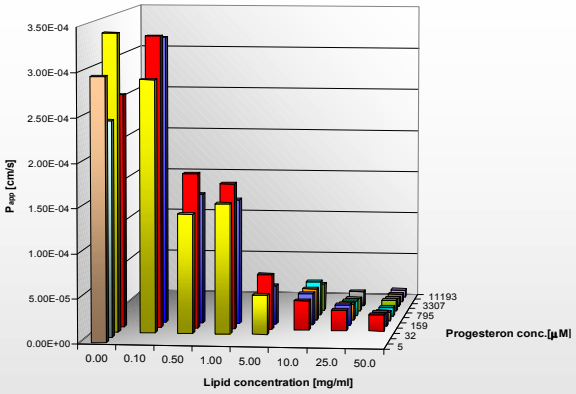
•Liposome-water distribution coefficient K was calculated with equilibrium dialysis experiments to be K=2242528. Free fraction of drug concentration was calculated using this value.

•A model for P_{app} was derived taking in account the membrane and diffusion boundary layer permeability coefficients P_m and P_{DBL} respectively. For the derivation was assumed that only free drug permeates the cell membrane.

•The model explained satisfactorily the experimental results indicating that for this drug permeation is controlled by free drug concentration.

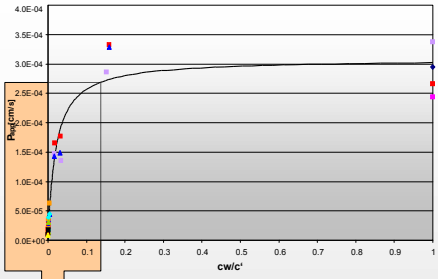
•Permeation was controlled mainly by the transport rate through diffusion boundary layer

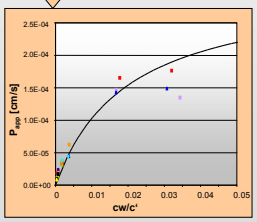
Influence of Lipid and drug concentration on P_{app}



For the liposomal formulations, P_{app} strongly decreased with increasing lipid concentration. Within the same lipid concentration P_{app} was independent of drug concentration in analogy to the aqueous solution. Each colour represents a different Progesterone concentration. This result may be explained by the assumption that only unbound drug can pass the cell monolayer. The majority of progesterone is distributed into the liposome membrane because of the high lipophilicity of this drug. With increasing lipid content in the formulation, the concentration of free progesterone decreases which leads to the decreasing P_{app} values.

Calculation of membrane and diffusion boundary layer permeability coefficients





The following equation was fitted to the experimental results with EasyFit

$$P_{app} = \frac{c_w/c'}{\frac{c_w/c'}{P_{DBL}} + \frac{1}{P_m}}$$

P is a Permeability coefficient. The indices app mean apparent, DBL diffusion boundary layer, m caco-2 cell membrane. The ratio c_w/c' is the free fraction of drug. cw is the free drug, c' the total drug concentration.

Permeability coefficients deduced from the fit

P_m	0.0154 cm/s
P_{DBL}	0.000308 cm/s
Final residual Value	$2.52*10^{-2}$

In the left upper diagram P_{app} is plotted against the free fraction of Progesterone. The lower diagram is an enlargement of the low value range.

Every symbol represents the calculated P_{app} of a bidirectional transport experiment. Same colour stands for the same total drug concentration (same as for the 3D-diagram). The drawn line represents the fitted model. Papp is independent of the used total drug concentration but strongly dependent on the liposome-water distribution of Progesterone.

Mathematical model for cellular transport

Transport from apical to basal

$$\frac{dC_{aAB}}{dt} = -P_{app} * (c_{aAB} - c_{cAB}) * Sm / Va + v_k * Sm / Va \quad \text{Eq. 1}$$

$$\frac{dC_{bAB}}{dt} = P_{app} * (c_{cAB} - c_{bAB}) * Sm / Vb \quad \text{Eq. 2}$$

$$\frac{dm_{cAB}}{dt} = P_{app} * (c_{aAB} - c_{cAB}) * Sm - vk * Sm - P_{app} * (c_{cAB} - c_{bAB}) * Sm \quad \text{Eq. 3}$$

Transport from basal to apical

$$\frac{dC_{aBA}}{dt} = P_{app} * (c_{cBA} - c_{aBA}) * Sm / Va + vk * Sm / Va \quad \text{Eq. 4}$$

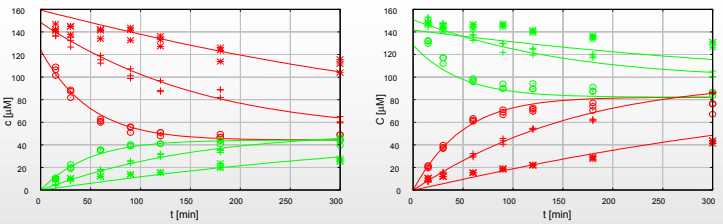
$$\frac{dC_{bBA}}{dt} = -P_{app} * (c_{bBA} - c_{cBA}) * Sm / Vb \quad \text{Eq. 5}$$

$$\frac{dm_{cBA}}{dt} = P_{app} * (c_{bBA} - c_{cBA}) * Sm - P_{app} * (c_{cBA} - c_{aBA}) * Sm - vk * Sm \quad \text{Eq. 6}$$

$$v_k = \frac{v_{max} * M_{cab}}{K + M_{cab}} \quad \text{Eq. 7}$$

P_{app} is the apparent permeability coefficient, Sm is the membrane surface area, Va is the volume of the apical compartment, Vb is the basal volume. Where c is concentration, the indices a, b, and c (lower case) denote the apical, basal and cellular compartment, respectively, the indices AB and BA (upper case) denote permeation in the apical-to-basal and the basal-to-apical direction, respectively. v_k expresses saturable apical efflux rate.

Transport Profiles with Different Lipid contents apical-basal (left) and basal- apical (right)



Symmetric transport of the drug was observed for the liposome formulations and the aqueous solution through Caco-2 cell monolayers, which was consistent with a passive transport of the used drug. Each symbol represents a measured concentration value of different transport studies with 160µM Progesterone and different liposome concentrations. o o represents 0.5mg lipids/ml, + + represents 5mg lipids/ml, * * 50mg lipids/ml. The apical concentrations are coloured in red, the basal in green. The drawed lines display the resulting fit to the mathematical model for cellular transport using EasyFit.

Literature

[1] Absorption of poorly water soluble drugs subject to apical efflux using phospholipids as solubilizers in the Caco-2 cell model, Susanne B. Kapitza, Bettina R. Michel, Peter van Hoogevest, Mathew L.S. Leigh and Georgios Imanidis, European Journal of Pharmaceutics and Biopharmaceutics *In Press, Accepted Manuscript, Available online 23 August 2006*